The Effect of Multiple Sequential Light Sources to Activate Aminolevulinic Acid in the Treatment of Actinic Keratoses: A Retrospective Study

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ABSTRACT

There is a lack of research regarding the sequential use of multiple light sources for topical 5-aminolevulinic acid activation in photodynamic therapy for actinic keratosis. This study evaluated 5-aminolevulinic acid-photodynamic therapy for actinic keratosis using blue light combined with red light, pulsed dye laser, and/or intense pulsed light in a retrospective fashion. Field-directed 5-aminolevulinic acid-photodynamic therapy was performed with blue light only, blue light + pulsed dye laser, blue light + intense pulsed light, blue light + pulsed dye laser + intense pulsed light, or blue light + red light + pulsed dye laser + intense pulsed light for nonhyperkeratotic actinic keratoses of face, scalp, or upper trunk. Blue light + intense pulsed light + pulsed dye laser produced greater patient-reported improvement in actinic keratoses than blue light or blue light + intense pulsed light and greater subject-reported improvement in overall skin quality than blue light + intense pulsed light. The addition of red light led to no further benefit in either outcome measure. Photodynamic therapy with multiple, sequential laser and light sources led to greater patient-graded improvement in actinic keratoses than that with a single light source (blue light), without significant differences in post-treatment adverse events. However, the small, widely disparate number of patients between groups and follow-up times between patients, as well as retrospective assessments based on subjective patient recall, severely limit the significance of these findings. Nevertheless, the results raise interesting questions regarding the use of multiple light and laser sources for photodynamic therapy of actinic keratoses and warrant further research with a prospective, randomized, controlled study. (*J Clin Aesthet Dermatol.* 2014;7(9):20–25.)

Actinic keratoses (AKs) are dysplastic epidermal neoplasms resulting from chronic cutaneous exposure to ultraviolet radiation, commonly found within photodamaged areas of the face, bald scalp, posterior neck, upper trunk, and dorsal upper extremities.[1] Risk factors for the development of AKs include older age, male gender, Fitzpatrick I and II skin types, proximity to the equator, immunosuppression, and cumulative exposure to sunlight, tanning beds, and/or psoralen + ultraviolet A light (PUVA).[1–3] The fact that 65 to 97 percent of squamous cell carcinomas develop from AKs or areas of field cancerization highlights the need for effective treatment of these lesions.[2]

Numerous options exist for the management of AKs (Table 1), each with their own risk-benefit profile. Photodynamic therapy (PDT) has gained popular support given its ability to treat large areas with prolonged recurrence-free periods, excellent cosmetic outcomes, and only modest morbidity, without the need for strict patient compliance.[4] PDT may also have the potential to decrease expression of early markers of cutaneous neoplasia (e.g., Ki-67 and p53), as demonstrated in multiple studies following methyl aminolevulinate PDT (MAL-PDT) using incoherent red light.[5,6] Complete response rates with PDT vary based on the area treated, number of sessions required, and the type of exogenous photosensitizer and light or laser source used, ranging from 50 to 90 percent.[6–14]

Topical PDT requires the interaction of an exogenous photosensitizer, an activating light source, and the presence of oxygen. The nonphotosensitizing prodrug 5-aminolevulinic acid (ALA) is preferentially absorbed by and metabolized within rapidly proliferating dysplastic keratinocytes, producing highly photoactive

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protoporphyrin IX (PpIX).[15,16] The methylated, more lipophilic derivative of ALA, MAL, may more selectively accumulate PpIX within premalignant cells.[17] The absorption spectrum of PpIX includes a maximal peak at 410nm (Soret band) and four smaller peaks (Q bands) from 500 to 630nm (Figure 1).[18] PpIX excitation with a light source of an appropriate wavelength produces cytotoxic singlet oxygen and other reactive oxygen species (ROS), with destruction of dysplastic epidermal cells as well as actinically damaged collagen fibers and subsequent neocollagenesis with fibroblast stimulation.[19–21]

Photoactivation of porphyrins with a single light source, including incoherent, continuous-wave red or blue light, pulsed-dye laser (PDL), or intense pulsed light (IPL), has been the foundation of traditional PDT.[22] Goldman and Atkin first proposed using PDT as field therapy for both clinical and subclinical AKs.[23] Although numerous studies have utilized PDT for AKs, there is a scarcity of literature describing the sequential use of multiple light and laser sources for photosensitizer activation. The anti-inflammatory and epidermal turnover properties of blue light may act in synergy with the deeper penetration of red light and the photothermal effects of pulsed lasers, leading to improved, more durable results.[19] Moreover, the sequential use of different light sources may guarantee that the multiple absorption peaks of PpIX are successfully targeted during treatment and that maximal photobleaching of porphyrins is achieved, which typically does not occur with the use of a single laser or light source.[24,25]

The aim of this nonblinded, multi-arm, retrospective study was to compare the safety and efficacy of ALA-PDT for actinic keratosis using blue light combined with red light, PDL, and/or IPL.

METHODS

Sixty-five patients (93 sessions) treated with field-directed ALA-PDT between 2001 and 2010 for nonhyperkeratotic AKs of face, scalp, and upper trunk were enrolled in this retrospective, single-center study. Nonrandomized treatments were performed with either blue light only, blue light + PDL, blue light + IPL, blue light + PDL + IPL, or blue light + red light + PDL + IPL by two board-certified dermatologists. All treatment areas were degreased with acetone and exfoliated with 5min of microdermabrasion prior to application of 20% ALA (Levulan Kerastick, Dusa Pharmaceuticals Inc., Wimington, Massachusetts), with 1h unoccluded incubation in a dimly lit room. A baby wipe was used to remove any residual ALA immediately prior to treatment.

All patients were exposed to 417nm blue light (BLU-U, DUSA Pharmaceuticals Inc.) for 16min 40s with a fluence dose of 10J/cm^2. If 630nm red light (Aktilite CL128, Photocure Inc., Princeton, New Jersey) was used in addition to blue light, simultaneous or sequential irradiation was performed for 8min with a fluence dose of 37J/cm^2. To increase patient comfort, cold-air cooling (Artek Air, Thermotek Inc., Flower Mound, Texas) was used during blue and/or red light exposure. PDL (Cynergy, Cynosure, Westford, MA) and IPL (Lumenis 1 or M22, Lumenis Inc., San Jose, CA), if performed, were always utilized prior to blue and red light sources. PDL spot-treatment of AKs used 2 passes with a 5-7 mm spot-size to deliver pulse durations of 10 to 40ms and fluences of 5 to 12J/cm^2. The Cynergy device required forced cold air cooling (Cryo 5, Cynosure, Westford, Massachusetts). Standard IPL treatment parameters based on patient skin type included mean double-pulse durations of 3.5ms with a delay of 10 to 30ms and fluences of 15 to 22J/cm^2, using a 560nm cutoff filter. In addition to the IPL's chilled 15x35mm sapphire crystal and a thick layer of optical coupling gel, periprocedural cold air

TABLE 1. Available treatment options for actinic keratoses
NSAIDs, nonsteroidal anti-inflammatory drugs

Topical	NSAIDs (diclofenac in hyaluronic gel) 5-fluorouracil Imiquimod, resiquimod Masoprocol
Oral	Retinoids
Chemical	Liquid nitrogen cryotherapy Photodynamic therapy Chemical peels (medium or greater depth)
Mechanical	Dermabrasion Nonablative laser resurfacing (1927nm fractional thulium fiber) Ablative laser resurfacing (CO_2, Erbium:YAG)

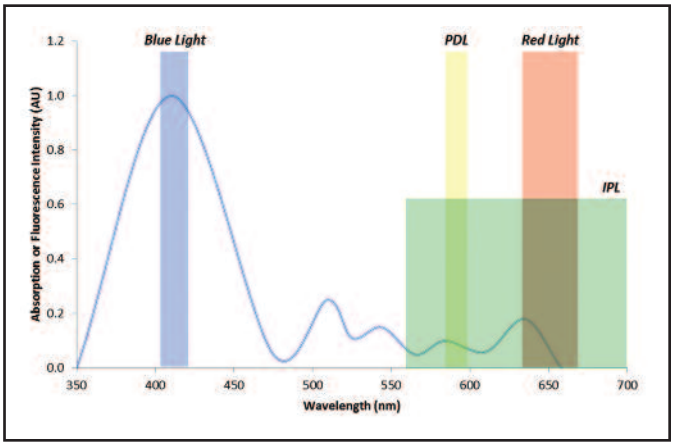

Figure 1. *In vivo* absorption spectrum for protoporphyrin IX with peaks at 405–415nm (Soret band) and 506–540nm, 572–582nm, and 628–635nm (Q bands).[18] Wavelengths of pertinent light sources are overlapped, including incoherent blue light (peak 417nm), incoherent red light (peak 635nm), pulsed-dye laser (PDL; 585-595nm), and intense pulsed light (IPL; 560–1200nm).

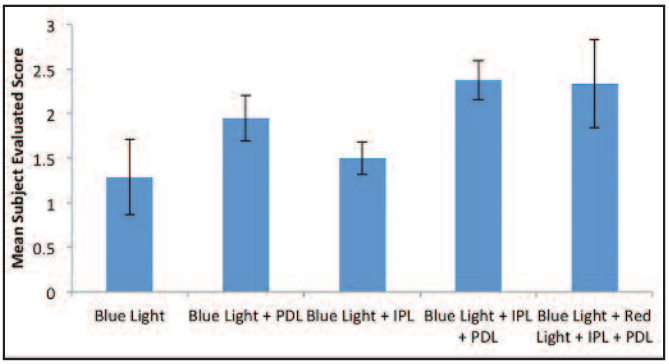

Figure 2. Mean improvement in actinic keratoses following photodynamic therapy. Blue light + IPL + PDL led to greater improvement in AKs than blue light only or blue light + IPL.

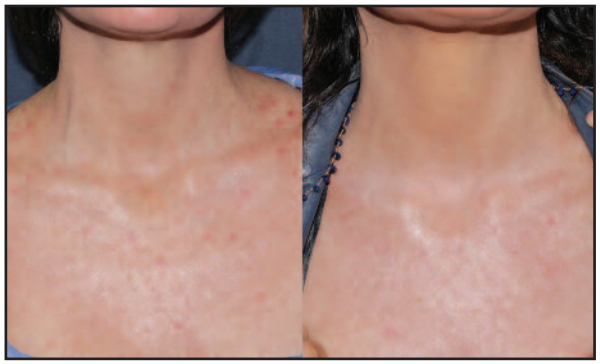

Figure 3. Upper chest (left) before treatment and (right) one month following one session of PDT with blue light, red light, PDL, and IPL.

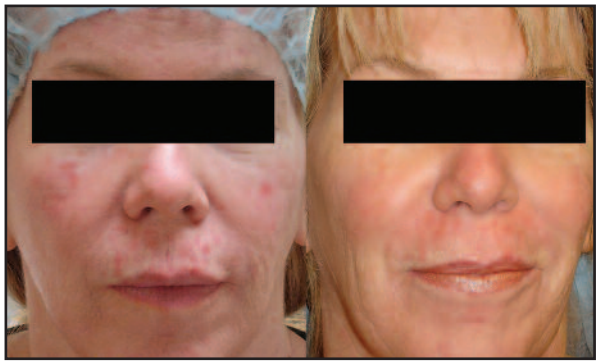

Figure 4. Full face (left) before treatment and (right) seven months following one session of PDT with blue light, PDL, and IPL.

cooling (Cryo 5) was used to increase patient comfort. The off-label use of these pulsed modalities for AKs was discussed with patients prior to every therapeutic session. Patients had a sunscreen (titanium dioxide or zinc oxide) applied immediately following treatment and were advised regarding strict sun protection of treated areas for 36h.

A prescripted telephone questionnaire was used to collect patient-reported outcome measures, including

TABLE 2. Subjects by treatment arm
PDL, pulsed dye laser. IPL, intense pulsed light

GROUP	TOTAL SUBJECTS/ TOTAL SESSIONS
Blue light	6/7
Blue light + PDL	16/24
Blue light + IPL	26/38
Blue light + IPL + PDL	12/18
Blue light + red light + IPL + PDL	5/6

prevalence of adverse events (peeling, acne, erythema, and pain), improvement in AKs, and improvement in overall skin quality. Follow-up was performed 1 to 8 years post-treatment for all patients. All outcome criteria were subjectively graded by patients using 4-point scales: 0=none, 1=mild, 2=moderate, 3=severe for adverse events and 0=none, 1=mild, 2=moderate/good, 3=excellent for improvement in AKs or overall skin quality. Patient willingness to undergo repeat treatment with ALA-PDT (if needed) was assessed with a 3-point scale (0=no, 1=yes, 2=undecided). Two-sample *t*-tests were subsequently used to compare parallel-group data.

RESULTS

Of the 40 total procedures, 46 percent were performed on male and 54 percent were performed on female patients. Patients had a mean of 1.4±0.8 (1–4) sessions (Table 2). The mean age of patients treated was 58.9 years. No standardized lesion count was performed at baseline or on follow-ups. Forty-five percent of patients stated that they would repeat the procedure, 39 percent stated that they would not, and 16 percent were undecided.

Mean degree of AK improvement was 1.8 (mild-to-moderate) among all patients. Patients treated with blue light + IPL + PDL reported greater improvement in their AKs than those treated with blue light only (p=0.020) and blue light + IPL (p=0.008; Figures 2–4). Mean improvement in overall skin quality was 1.7 (mild-to-moderate) among all AK patients treated with ALA-PDT. Patients treated with blue light + IPL + PDL reported greater improvement in overall skin quality than those treated with blue light + IPL (p=0.045; Figure 5).

Sixty-three percent of patients reported some degree of peeling post procedure, 13 percent reported acne outbreaks, 91 percent reported erythema, and 71 percent reported pain (Figure 6). Patients treated with blue light + PDL reported a higher average rate of erythema than those treated with blue light + IPL + PDL (p=0.033) and higher rates of pain than those treated with blue light + red light + IPL + PDL (p=0.035). Patients treated with blue light + IPL reported greater peeling than those treated with blue light + red light + IPL + PDL (p=0.032) and greater erythema than those treated with blue light + IPL + PDL (p=0.002) and blue light + red light + IPL + PDL (p=0.023). Patients treated with blue light + IPL reported greater acne flares post-procedure than those treated with blue light + red light + IPL + PDL (p=0.022).

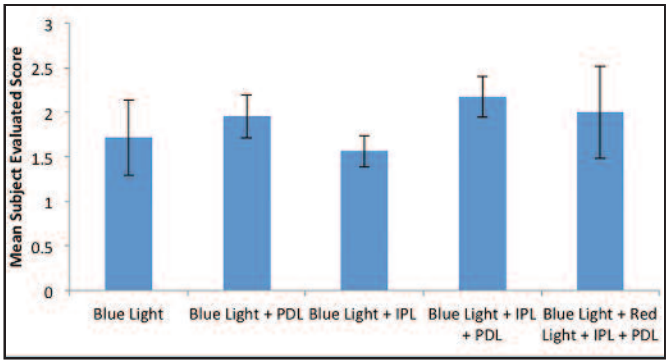

Figure 5. Mean improvement in overall skin quality following photodynamic therapy of actinic keratoses. Blue light + IPL + PDL led to greater improvement in overall skin quality than blue light + IPL.

DISCUSSION

The authors' retrospective, parallel-group study has demonstrated greater efficacy toward the treatment of AKs using three sequential light and laser sources, blue light + PDL + IPL, compared to blue light only (p=0.020) or blue light + IPL (p=0.008) based on subjective, patient-graded results. Blue light + PDL + IPL also led to greater mean improvement in overall skin quality than blue light + IPL (p=0.045). The addition of red light to the other devices led to no further improvement in both outcome measures, perhaps because a maximal photobleaching threshold is achieved with the use of the other three sequential light and laser sources. However, the small sample size in the red light + blue light + PDL + IPL group may account for the lack of significant difference.

Increased numbers of light and laser sources were also not associated with increased or worsened adverse events. On the other hand, blue light + red light + PDL + IPL led to significantly less erythema (p=0.023), peeling (p=0.032), and acne flares (p=0.022) than blue light + IPL and significantly less pain than blue light + PDL (p=0.035). Blue light + PDL + IPL also caused significantly less erythema than blue light + IPL (p=0.002) or PDL (p=0.033).

Nevertheless, the relatively small number of subjects in the blue light and red light + blue light + PDL + IPL groups and the overall disparate patient numbers between groups limits statistical significance between multiple, increasing number of modalities for the treatment of actinic keratoses. The authors' single-center study also had several other major limitations, including inconsistent follow-up times between patients and a lack of internal clinical controls or randomization. Although patients who could not recall or had substantial difficulty recalling having had the procedure were excluded, recall bias may still be a significant factor in the results.

PDT for the treatment of clinical AKs and subclinical lesions within background severely photodamaged areas has been studied extensively. Two multicenter, Phase 3 clinical trials evaluated 243 subjects with 4 to 15 nonhyperkeratotic AKs of the face and scalp, with subjects randomized to a single session of 20% ALA or vehicle, followed by blue light (10J/cm^2, 16min 40s).[26,27] At 12 weeks post-treatment, 73 percent of subjects with ALA had a complete response (100% clearance) compared to eight percent with vehicle. Ninety-one percent of AKs cleared with ALA at the same timepoint compared to 25 percent with vehicle. PDT has likewise been shown to produce histological evidence of neocollagenesis, epidermal-dermal remodeling, and improvement in atypia. A study of 26 patients with facial AKs and photodamage also showed improved atypia grade (1.46 to 0.69, p<0.001) and extent (0.49 to 0.26, p<0.001) at three months relative to baseline following three sessions of MAL with red light.[6] Twenty-two subjects with biopsy-proven AKs of the face and scalp had significant reductions in basal keratinocyte dysplasia and elastosis (p<0.005) six weeks after a single treatment of MAL with red light. Ki-67 overexpression and p53 expression decreased in 77 percent (p<0.0001) and 55 percent (p<0.002) of subjects, respectively [5].

Only one prospective study has investigated the sequential use of multiple laser or light sources for the photodynamic management of AKs. A single session of MAL-PDT with either red or blue light was used to treat moderate-to-severe photodamage of the face, scalp, or upper trunk in a split-site study of 18 patients.[28] The majority of patients also had concurrent spot-treatment with PDL and/or field-directed treatment with IPL to affected areas. At one-month follow-up, there was no statistically significant difference in evaluator-graded improvement in AKs between red and blue light groups (p=1.00), and neither led to a statistically significant reduction relative to baseline.

The sequential use of different light and laser sources still likely takes advantage of their distinct mechanisms of action, mitigates their individual weaknesses, and allows for the targeting of multiple porphyrin absorption peaks concurrently, maximizing photobleaching and leading to improved overall efficacy. Although the authors' results did not show any additional benefit from the combination of red and blue light, the synergy of these light sources may

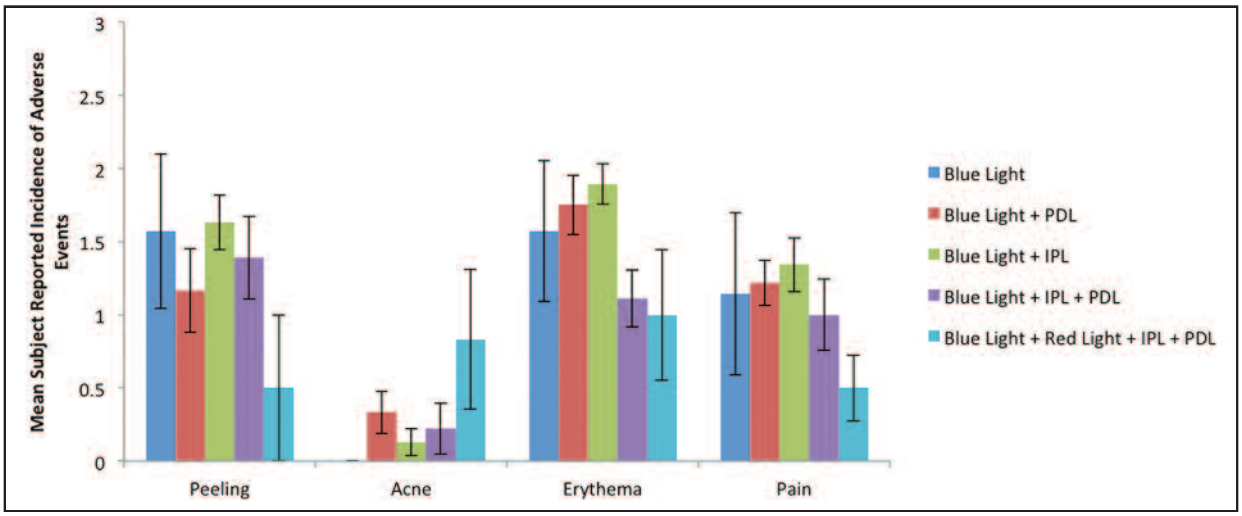

Figure 6. Post-procedure adverse events. Blue light + red light + IPL + PDL was associated with less pain than blue light + PDL and less acne flares, erythema, and peeling than blue light + IPL. Blue light + IPL + PDL led to less erythema than blue light + IPL or blue light + PDL.

likely further improve photodynamic results by pairing superior PpIX absorption at 417nm with greater depth of penetration and decreased superficial melanin absorption at 635nm, respectively, allowing for the targeting of deeper dermal structures such as sebaceous glands.[29] IPL and broad-spectrum, incoherent light sources may also potentiate photodynamic effects by photoactivating porphyrin degradation products.[30] Despite the controversy that exists regarding the ability of PDL and IPL to modestly photoactivate endogenous porphyrins, given the likelihood of oxygen depletion during their brief (millisecond) pulse durations, non-oxygen-dependent porphyrin reactions and highly efficient, rapidly produced singlet oxygen may play a compensatory role.[31] PDL and IPL also both lead to nonablative photorejuvenation that safely and effectively improves the aesthetic appearance of aged, photodamaged skin with low side effect profiles and minimal patient discomfort or post-treatment downtime.[32,33] The greater level of patient-graded improvement in AKs noted in the study when blue light was combined with PDL and IPL may likewise further contribute to recall bias, given that patients may have reported improvement in AKs, when in fact they had improvement in photodamage, dyspigmentation, and skin texture.

CONCLUSION

Although PDT with multiple, sequential laser and light sources (blue light + PDL + IPL) led to greater patient-reported improvement in AKs than a single light source (blue light) or blue light + IPL without increased adverse events, the major statistical flaws of this study notably limit the significance and reliability of the retrospective data. Nevertheless, these findings warrant evaluation with a prospective randomized-controlled study.

REFERENCES

1. Goldberg LH, Mamelak AJ. Review of actinic keratosis. Part I: etiology, epidemiology and clinical presentation. J *Drugs Dermatol*. 2010;9:1125–1132.
2. Rosen T, Lebwohl MG. Prevalence and awareness of actinic keratosis: barriers and opportunities. *J Am Acad Dermatol*. 2013;68:S2–S9.
3. Basset-Seguin N, Baumann Conzett K, Gerritsen MJP, et al. Photodynamic therapy for actinic keratosis in organ transplant patients. *J Eur Acad Dermatol Venereol*. 2013;27:57–66.
4. Tierney E, Barker A, Ahdout J, et al. Photodynamic therapy for the treatment of cutaneous neoplasia, inflammatory disorders, and photoaging. *Dermatol Surg*. 2009;35:725–746.
5. Bagazgoitia L, Cuevas Santos J, Juarranz A, Jaén P. Photodynamic therapy reduces the histological features of actinic damage and the expression of early oncogenic markers. *Br J Dermatol*. 2011;165:144–151.
6. Szeimies RM, Torezan L, Niwa A, et al. Clinical, histopathological and immunohistochemical assessment of human skin field cancerization before and after photodynamic therapy. *Br J Dermatol*. 2012;167:150–159.
7. Tschen EH, Wong DS, Pariser DM, et al. Photodynamic therapy using aminolaevulinic acid for patients with nonhyperkeratotic actinic keratoses of the face and scalp: phase IV multicentre clinical trial with 12-month follow up. *Br J Dermatol*. 2006;155:1262–1269.
8. Szeimies RM, Karrer S, Sauerwald A, Landthaler M. Photodynamic therapy with topical application of 5-aminolevulinic acid in the treatment of actinic keratoses: an initial clinical study. *Dermatology* (Basel). 1996;192:246–251.
9. Zane C, Capezzera R, Sala R, et al. Clinical and echographic analysis of photodynamic therapy using methylaminolevulinate as sensitizer in the treatment of photodamaged facial skin.

Lasers Surg Med. 2007;39:203–209.
10. Piaserico S, Belloni Fortina A, Rigotti P, et al. Topical photodynamic therapy of actinic keratosis in renal transplant recipients. *Transplant Proc*. 2007;39:1847–1850.
11. Annemans L, Caekelbergh K, Roelandts R, et al. Real-life practice study of the clinical outcome and cost-effectiveness of photodynamic therapy using methyl aminolevulinate (MAL-PDT) in the management of actinic keratosis and basal cell carcinoma. *Eur J Dermatol*. 2008;18:539–546.
12. Fai D, Arpaia N, Romano I, et al. Methyl-aminolevulinate photodynamic therapy for the treatment of actinic keratoses and non-melanoma skin cancers: a retrospective analysis of response in 462 patients. *G Ital Dermatol Venereol*. 2009;144:281–285.
13. Kleinpenning MM, van de Kerkhof PCM, Gerritsen RMJP. The clinical efficacy of topical methyl-aminolevulinate photodynamic therapy in moderate to severe actinic keratoses of the face and scalp. *J Dermatolog Treat*. 2010;21:252–257.
14. Gilbert DJ. Treatment of actinic keratoses with sequential combination of 5-fluorouracil and photodynamic therapy. *J Drugs Dermatol*. 2005;4:161–163.
15. Gold MH, Goldman MP. 5-aminolevulinic acid photodynamic therapy: where we have been and where we are going. *Dermatol Surg*. 2004;30:1077–1083.
16. Divaris DX, Kennedy JC, Pottier RH. Phototoxic damage to sebaceous glands and hair follicles of mice after systemic administration of 5-aminolevulinic acid correlates with localized protoporphyrin IX fluorescence. *Am J Pathol*. 1990;136:891–897.
17. Lehmann P. Methyl aminolaevulinate-photodynamic therapy: a review of clinical trials in the treatment of actinic keratoses and nonmelanoma skin cancer. *Br J Dermatol*. 2007;156:793–801.
18. Pottier RH, Chow YF, LaPlante JP, et al. Non-invasive technique for obtaining fluorescence excitation and emission spectra in vivo. *Photochem Photobiol*. 1986;44:679–687.
19. Sakamoto FH, Lopes JD, Anderson RR. Photodynamic therapy for acne vulgaris: a critical review from basics to clinical practice: part I. Acne vulgaris: when and why consider photodynamic therapy? *J Am Acad Dermatol*. 2010;63:183–193.
20. Lubart R, Friedmann H, Lavie R, et al. A reasonable mechanism for visible light-induced skin rejuvenation. *Lasers Med Sci*. 2007;22:1–3.
21. Nakaseko H, Kobayashi M, Akita Y, et al. Histological changes and involvement of apoptosis after photodynamic therapy for actinic keratoses. *Br J Dermatol*. 2003;148:122–127.
22. Touma DJ, Gilchrest BA. Topical photodynamic therapy: a new tool in cosmetic dermatology. *Sem Cutan Med Surg*. 2003;22:124–130.
23. Goldman M, Atkin D. ALA/PDT in the treatment of actinic keratosis: spot versus confluent therapy. *J Cosmet Laser Ther*. 2003;5:107–110.
24. Alexiades-Armenakas M. Intense pulsed light-mediated photodynamic therapy. *J Drugs Dermatol*. 2005;4:657–658.
25. Togsverd-Bo K, Idorn LW, Philipsen PA, et al. Protoporphyrin IX formation and photobleaching in different layers of normal human skin: methyl- and hexylaminolevulinate and different light sources. *Exp Dermatol*. 2012;21:745–750.
26. Gold MH. The evolving role of aminolevulinic acid hydrochloride with photodynamic therapy in photoaging. *Cutis*. 2002;69:8–13.
27. Kalisiak MS, Rao J. Photodynamic therapy for actinic keratoses. *Dermatol Clin*. 2007;25:15–23.
28. Palm MD, Goldman MP. Safety and efficacy comparison of blue versus red light sources for photodynamic therapy using methyl aminolevulinate in photodamaged skin. *J Drugs Dermatol*. 2011;10:53–60.
29. Kosaka S, Miyoshi N, Akilov OE, et al. Targeting of sebaceous glands by δ-aminolevulinic acid-based photodynamic therapy: an in vivo study. *Lasers Surg Med*. 2011;43:376–381.
30. Brancaleon L, Moseley H. Laser and non-laser light sources for photodynamic therapy. *Lasers Med Sci*. 2002;17:173–186.
31. Strasswimmer J, Grande DJ. Do pulsed lasers produce an effective photodynamic therapy response? *Lasers Surg Med*. 2006;38:22–25.
32. Sadick NS, Weiss R, Kilmer S, Bitter P. Photorejuvenation with intense pulsed light: results of a multi-center study. *J Drugs Dermatol*. 2004;3:41–49.
33. Galeckas KJ, Ross EV, Uebelhoer NS. A pulsed dye laser with a 10-mm beam diameter and a pigmented lesion window for purpura-free photorejuvenation. *Dermatol Surg*. 2008;34:308–313.